AD_____

Award Number: W81XWH-08-1-0503

TITLE: Noninvasive Subharmonic Pressure Estimation for Monitoring Breast Cancer Response to Neoadjuvant Therapy

PRINCIPAL INVESTIGATOR: Flemming Forsberg, Ph.D.

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, Pennsylvania 19107

REPORT DATE: September 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| 01-09-2010 | Annual | 1 SEP 2009-31 AUG 2010 |

**4. TITLE AND SUBTITLE**
Noninvasive Subharmonic Pressure Estimation for Monitoring Breast Cancer Response to Neoadjuvant Therapy

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-08-1-0503

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Dr. Flemming Forsberg

E-Mail: cnazario@rcm.upr.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)**
Thomas Jefferson University
Philadelphia, Pennsylvania 19107

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
Neoadjuvant chemotherapy is currently the standard of care for locally advanced breast cancer (LABC). Monitoring tumor response is advantageous for patients. This project aims at establishing noninvasive monitoring of neoadjuvant chemotherapy in the breast using subharmonic aided pressure estimation (SHAPE) to estimate the interstitial fluid pressure (IFP) in LABC. To date, in vitro experiments with the ultrasound contrast agent Definity have showed an inverse linear relationship between the change in subharmonic amplitude and hydrostatic pressure ($r2 = 0.79–0.99$, $p < 0.01$) over the pressure range associated with breast tumors (0 – 50 mmHg). Moreover, software for analyzing RF data from a Sonix RP scanner to produce SHAPE pressure estimates has been successfully optimized and submitted for publication.In vivo proof of concept for SHAPE as a noninvasive monitor of IFP ($r2 > 0.81$, $p < 0.01$) has been provided based on a swine model. However, difficulty with standing waves in the in vitro setup has delayed the project by approximately 9 - 12 months.

**15. SUBJECT TERMS**
Breast Cancer, Ultrasound Imaging, Ultrasound Contrast Agent, Pressure Estimation, Neoadjuvant chemotherapy

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 17 | **19b. TELEPHONE NUMBER** *(include area code)* |

## 3 TABLE OF CONTENTS

4 INTRODUCTION ..... 4
5 BODY ..... 5
5.1 Methods ..... 6
5.2 Results and Discussion ..... 7
6 KEY RESEARCH ACCOMPLISHMENTS ..... 10
7 REPORTABLE OUTCOMES ..... 11
8 CONCLUSIONS ..... 13
9 REFERENCES ..... 14
Appendix I ..... 16

# 4 INTRODUCTION

In the United States, close to 5 – 20 % of newly diagnosed breast cancer and 10 – 30% of all primary breast cancer is diagnosed as locally advanced breast cancer (LABC) [1, 2]. Neoadjuvant chemotherapy (systemic preoperative chemotherapy) is currently the standard of care for LABC [3, 4]. When compared with adjuvant chemotherapy (postoperative therapy), neoadjuvant chemotherapy yields similar results for both overall survival (70% for both) and disease-free survival (53% adjuvant, 55% neoadjuvant) [5]. Thus, the postponement of surgery does not affect the outcome of the treatment [5, 6]. In addition, neoadjuvant chemotherapy offers considerable benefits to the patient as the treatment can shrink the tumor and even in some cases offer complete pathologic response [3, 7]. This reduction in tumor size increases the possibility of breast conservation [3, 5-7]. Maximizing the conservation of breast tissue can be of great personal importance for the self-esteem and quality of living of the patient [6]. Neoadjuvant chemotherapy can also offer an early indication of the patient's response to chemotherapy. Consequently, monitoring tumor response to neoadjuvant therapy gives the possibility of adjusting the treatment if the patient is responding poorly or not at all resulting in substantial advantages for the patient [3, 6]. This project aims at establishing noninvasive monitoring of neoadjuvant chemotherapy in the breast using subharmonic aided pressure estimation (SHAPE; U.S. Patent 6,302,845).

Generally interstitial fluid pressure (IFP) is 10-30 mmHg higher in cancerous tissue than in normal tissue although values of up to 60 mmHg have been recorded [8, 9]. Similarly, IFP in breast cancer tumors has been shown to be higher than that of surrounding breast tissue [9]. This increase is believed to be due to vascularity, fibrosis and difference in the interstitial matrix in tumors and it can result in poor transport of therapeutic drugs to tumors [8]. Taghian et al. used a wick-in-needle technique to monitor the IFP of breast cancer before and after neoadjuvant chemotherapy with two drugs used consecutively [10]. When used as a first drug Paclitaxel decreased the IFP by 36% ( $p=0.02$) whereas with Doxorubicin as a first drug there was only 8% reduction ($p=0.41$). As this was a hypothesis-generating study they did not show any outcome related to the relationship between IFP and therapy response [10]. However, the level of IFP has been shown to predict disease free survival for cervix cancer (34% disease free survival (DFS) if IFP > 19 mmHg, 68% DFS if IFP < 19 mmHg ($p = 0.002$ )) [11]. Thus, the level of interstitial fluid pressure (IFP) in breast cancer tumors could potentially be used to monitor the response to neoadjuvant chemotherapy.

Contrast agents have been used for two decades to improve visualization in ultrasound (US) imaging as they enhance the difference in reflectivity between tissues [12]. Because of the difference in compressibility between the medium and the microbubble any changes in pressure induce changes in the size of the microbubble [13]. This in turn affects the reflectivity and resonant frequency of the bubble [13, 14]. In subharmonic imaging (SHI) pulses are transmitted at a frequency $f_0$ and the echoes are received at half that frequency $f_0/2$. SHI has been showed to be a feasible option for contrast enhanced imaging due to subharmonic generation by contrast agents and limited subharmonic generation in tissues [15]. Our group came up with a novel technique, SHAPE, utilizing

microbubbles and the subharmonic amplitude of the scattered signal [13]. We showed that there is a linear relationship between the hydrostatic pressure and the subharmonic amplitude. We propose the use of SHAPE to monitor treatment response by noninvasively measuring the IFP in breast tumors. This offers several benefits to the patient. As opposed to the wick-in-needle technique SHAPE is noninvasive and does not inflict pain. Furthermore, it allows for an early indication of responders vs. non-responders and thereby makes adjustments to therapy easier. Moreover, SHAPE has been shown to have a favorable signal-to-noise ratio so the subharmonic amplitude is not affected by background noise [13].

The optimal contrast agent and acoustic parameters for SHAPE will be established using *in vitro* pulse-echo measurements. The SHAPE algorithm will then be designed and implemented on a commercial, state-of-the-art US scanner for *in vivo* IFP measurements. A similar algorithm has already been set up for cardiac SHAPE and thus only a few adjustments need to be made to implement SHAPE for breast tumors making this very cost-effective. The *in vivo* experiments will be twofold. First, athymic, nude, female rats will be implanted with SKBR3, MCF-7 or BT474 human breast cancer cells and SHAPE used to measure IFP and calibrated by comparing the SHAPE results to IFP measurements obtained with an invasive, intra-compartmental pressure monitor as the gold standard. After calibration, human xenograft breast tumors in athymic, nude, female rats will be used to evaluate the ability of SHAPE to track changes in IFP by studying before and after administration of a chemotherapy agent (paclitaxel).

Our group has proposed that SHAPE and contrast enhanced US imaging can be used to measure the IFP in LABC tumors, thus, making it possible to noninvasively monitor the tumor response to neoadjuvant chemotherapy. This method would be a considerable improvement from the wick-in-needle technique currently used for IFP measurements in LABC and allow for individualized treatments options.

## 5 BODY

The hypothesis of this project is that IFP in breast tumors can be measured noninvasively using SHAPE and contrast enhanced US thus improving the monitoring of neoadjuvant chemotherapy. To investigate this prospect, *in vitro* pulse-echo experiments will be conducted to investigate this prospect and find the optimal contrast agent for SHAPE. These results will then be used to implement SHAPE on a commercial scanner. The scanner will be used for *in vivo* studies on 201 rats with tumor xenografts in order to calibrate and evaluate SHAPE's ability to monitor response to neoadjuvant chemotherapy. The specific tasks of the project (as presented in the original Statement of Work) can be found in Appendix I.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

### *5.1 Methods*

*In vitro experiments*

Last year a pulse-echo system was constructed to test different types of contrast agents for use with high frequency SHAPE. The subharmonic amplitude at different static pressures was measured using a sealed water tank capable of withstanding pressure changes over 200 mmHg. Single element transducers with center frequencies of 4 to 12 MHz were used as transmitter and receiver. The pressure inside the tank was monitored by a pressure gauge (OMEGA Engineering, Stamford, CT). However, the acoustic pressures required to obtain reasonable subharmonic signals was very high (0.9 – 1.2 MPa). When further experiments were conducted at lower acoustic pressures (0.5 to 0.8 MPa) and/or a higher transmit frequencies (10 – 12 MHz), we discovered that the attenuation of the acoustic window of the water tank markedly influenced results (i.e., reduced or eliminated the subharmonic signal components). This was because the water tank employed was originally designed to withstand pressures up to 200 mmHg (i.e., cardiac pressures) rather than the smaller pressures encountered in breast tumor IFPs (up to 50 mmHg).

Hence it was decided to repeat the static pressure measurements using a more suitable water tank. Small (10 ml) OptiCell chambers (Nunc, Rochester, NY) have been used successfully to investigate interactions between contrast bubbles, ultrasound and cancer cells [16] and these were, therefore, selected as the new container for static pressure tests. The OptiCell was submerged into a larger water tank and 0.2 ml/l of Definity (Lantheus Medical Imaging, N Billerica, MA) injected. RF signals were acquired with a Sonix RP ultrasound scanner (Ultrasonix, Richmond, BC, Canada) using a high frequency, linear array and compared to an invasive (needle-in-wick) pressure monitor (Stryker, Berkshire, UK) as the reference standard.

A 2.5 cm tissue mimicking phantom was placed between the probe and the OptiCell chamber to simulate tissue attenuation. Two different transmit frequencies of 6.7 and 10 MHz were considered. Contrast echoes were received at half the transmit frequencies (i.e., 3.35 and 5.0 MHz). The acoustic output power was varied from 0 to -20 dB for a 0 mmHg hydrostatic pressure in order to establish the optimal sensitivity for SHAPE. Then the chamber pressure was varied from 0 to 50 mmHg to simulate IFP in tumors and acoustic pressure varied from -4 to -14dB. After data retrieval the amplitude of the subharmonic signal component was extracted using MATLAB 7.0.4 (Mathworks, Natick, MA). Three measurements were acquired at each setting and linear regression analysis used to determine the relationship between hydrostatic pressure and change in subharmonic amplitude. All statistical analyses were conducted using Stata 9.0 (Stata Corporation, College Station, TX).

Moreover, a novel, simulation model of the dynamics of an encapsulated microbubble contrast agent, developed as part of a previous DOD supported project [17], was modified in order to account for ambient pressure variations and different shell parameters to establish the optimal contrast microbubble for SHAPE. A nonlinear extension of the original viscoelastic model was pursued by considering a quadratic

elasticity model where the interfacial elasticity vary linearly with area fraction as well as an exponent.

*In vivo experiments*
Our group has worked in partnership with Ultrasonix Medical Corporation to implement SHAPE for cardiac use on a state-of-the-art commercial scanner Sonix RP (Ultrasonix Medical Corporation, Richmond, BC, Canada) with a phased array (PA4-2). Several experiments have been carried out in canines to investigate cardiac SHAPE supported by funding from the AHA. RF data from these experiments was analyzed off-line using Matlab. The software developed and optimized by our group for this analysis is not site-specific and will also be used to analyze the data we will acquire from *in vivo* breast SHAPE in rats as part of this project.

Finally, an opportunity to provide a proof-of-concept of the use of SHAPE for estimating IFP and test the invasive (needle based) Stryker pressure monitoring system (the reference standard) presented itself. As part of an ongoing NIH study, a unique, naturally occurring tumor model, the Sinclair swine with melanoma, was being studied. We obtained IFP measurements from the tumors and surrounding tissue using the Stryker needle based system, which provided us with a chance to assess the dependence of this technique on the angle between the needle and the tissue. Moreover, subharmonic signals were acquired during an infusion of Definity (7.5 ml/l/min) with the Sonix RP and a linear array. Data were obtained at 6.7 and 10 MHz (i.e., subharmonic frequencies of 3.75 and 5.0 MHz, respectively) with acoustic outputs of -4 and -8 dB. Five (5) swine were studied (one melanoma per swine) at no cost to this project.

### *5.2 Results and Discussion*

*In vitro experiments*
Over the pressure range of 0 – 50 mmHg (simulating the IFP in breast tumors) OptiCell measurements with Definity showed an inverse linear relationship between the change in subharmonic amplitude and hydrostatic pressure ($r^2 = 0.79–0.99$, $p < 0.01$). This is consistent with previous results reported by our group [13] and these efforts represent the partial fulfillment of tasks 1a, 1d and 1f in the original Statement of Work (SOW). An example of the subharmonic amplitude for Sonazoid at 0 mmHg and 47 mmHg can be seen in Figure 1 (transmitting frequency 7.5 MHz and acoustic pressure 0.7 MPa). However, the decrease in subharmonic amplitudes recorded for pressure changes from 0 to 50 mm Hg varied from 13.7 to 22.9 dB and 16.4 to 21.8 dB (depending on acoustic pressures) for 6.7 and 10 MHz transmission frequencies, respectively. These levels of subharmonic amplitude variation were markedly higher than our previous results obtained over a much larger pressure range (up to 200 mmHg) [13, 18-19].

After some extensive testing, we discovered that standing waves markedly influenced results and made measurements impossible to reproduce. Hence, our use of the OptiCell setup had to be abandoned and a completely new tank (with extra acoustic absorbers incorporated) had to be designed. The new water tank is currently being built, but this issue has delayed the project further by another approximately 4 months. Thus, the

project is approximately 9 to 12 months behind schedule and we, therefore, intend to request a one year no cost extension.

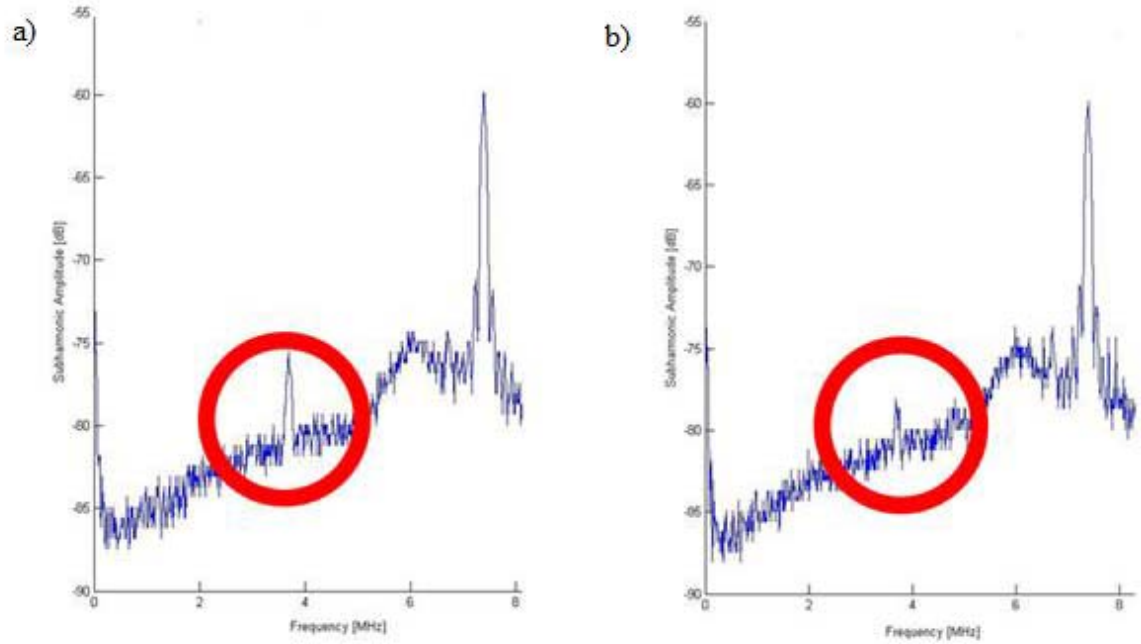


***Figure 1.*** *Comparison of the subharmonic amplitude (circle) measured with Sonazoid at a) 0 mmHg and b) 47 mmHg pressures.*

The previously developed simulation model of the dynamics of an encapsulated microbubble contrast agent was modified to include nonlinear extensions of the viscoelasticity and this model has now been published [20]. The intent was to better account for the experimentally observed changes in subharmonic signal amplitudes as a function of hydrostatic pressures. The model showed that the determining parameter of subharmonic response is the ratio of the excitation frequency to the resonance frequency. Changing the ambient pressure changes the resonance frequency and thereby the frequency ratio. For different acoustic excitation pressure levels, changing ambient pressure can either increase or decrease the subharmonic response depending on this ratio. For some range of parameters, the variation is far more complicated. This behavior is clearly at odds with the experimental observations mentioned above. These discrepancies may be due to encapsulation buckling or strain softening in describing nonlinear oscillations, specifically the subharmonic response [20]. This investigation has been submitted for publication [21]. This constitutes the continuation of tasks 1b, 1c and 1e.

*In vivo experiments*

Software to analyze RF data from the Sonix RP scanner has been improved and the best method to extract the subharmonic signal components from the frequency spectrum has been established. This software is applicable to RF data acquired from any tisssues

(including breast tumors). However, due to the availability of canine cardiac data (obtained with funds from other sources, but processed with funds form this grant), our initial work focused on cardiac SHAPE pressure estimates [22]. Briefly, the unprocessed RF data for each accumulated pulse (Figure 2A) was transformed to the Fourier domain and the subharmonic signal amplitude (at half the fundamental frequency - Figure 2B) was extracted as the average signal in a 40 % bandwidth around the subharmonic frequency (i.e., here 1.25 MHz). The extracted subharmonic signals from all pulses were processed using a moving average filter to eliminate noise spikes. The range of the subharmonic signal (i.e., the difference between maximum and minimum subharmonic amplitude) was compared from each pulse contour (after eliminating excessively noisy pulses) for each incident acoustic pressure. Since the pressure tracking using microbubbles is an incident acoustic pressure dependent phenomenon, the incident acoustic pressure with maximum stable subharmonic range was then selected for LV pressure tracking as shown in Figure 2C. Results from four canines indicate that an overall resolution on the order of 0.19 to 5.48 mmHg can be obtained for diastolic left ventricular pressures if the aortic pulse pressure values are known; otherwise resolution on the order of 0.64 to 8.98 mmHg can be obtained relative to an average calibration standard [23]. Moreover, a patent application has been submitted based on this development effort. Thus, considerable time has been spent on optimizing this software and this effort represents the conclusion of task 2b.

A unique opportunity to test the Stryker pressure monitoring system and provide proof-of-concept of the use of SHAPE for estimating IFP was pursued in the Sinclair swine melanoma model. Measurements were only obtained in three swine, because of technical difficulties (and only at 10 MHz in one of those animals, due to time constraints). At 6.7 MHz neither of the 2 tumors studied showed a statistically significant relationship between pressure and subharmonic signals ($p = 0.2$). Most likely this was due to the L14-5 probe having too high a bandwidth and the L9-4 linear array was, therefore, selected for the rat xenograft studies of task 3. Conversely, at 10 MHz one tumor showed a subharmonic decrease of 11.3 dB ($r^2 = 0.90$, $p < 0.01$) and 9.7 dB ($r^2 = 0.82$, $p < 0.01$) over 15 mmHg for acoustic output powers of -4 and -8 dB respectively (Figure 3). The second animal showed a decrease of 7.3 dB ($r^2 = 0.89$, $p < 0.01$) and 13.2 dB ($r^2 = 0.98$, $p < 0.01$) over 53 mmHg of variation (at -4 and -8 dB, respectively). This difference between tissue and tumor IFP is rather large and may be caused by the sensitivity of the Stryker system to the angle between the needle and the tissue being studied (this issue is currently being investigated further). The last melanoma swine had a pressure differential of 33 mmHg between the tumor and normal tissue with a corresponding 6.7 dB decrease in subharmonic signal amplitude at a -8 dB acoustic power ($r^2 = 0.92$, $p < 0.04$). In conclusion, *in vivo* proof of concept for SHAPE as a noninvasive monitor of IFP has been provided; albeit still based on a very small sample size and with relatively large subject-to-subject variation.

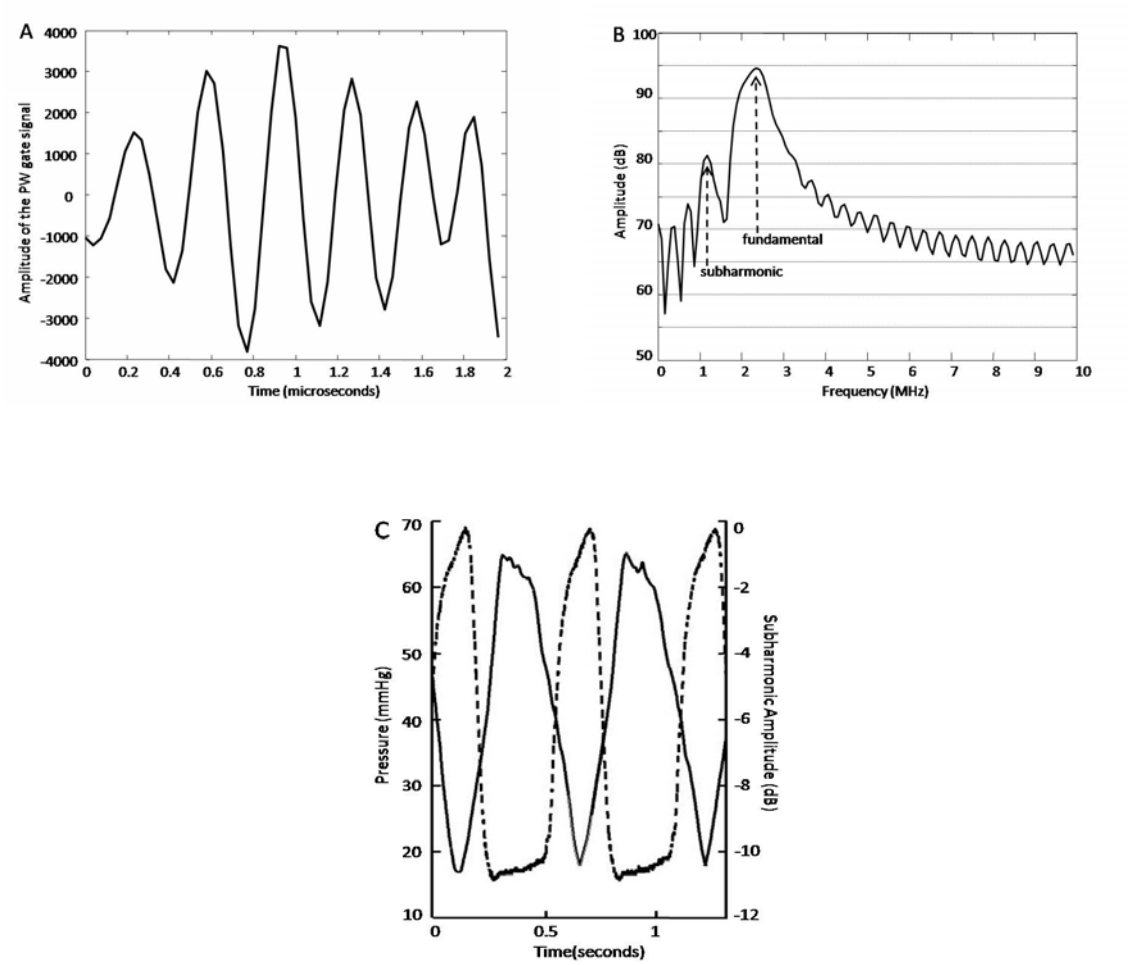


***Figure 2**: Steps involved in extracting and processing the subharmonic signal for SHAPE. A typical signal obtained with pulsed Doppler (A) and the frequency domain representation (B) of the signal in (A). The processed subharmonic signal from all the pulses (solid line) and the pressure catheter data (dotted line) are shown in (C). Note the inverse relationship between the subharmonic signal and the pressure obtained via the pressure catheter (in agreement with our previously published in vitro results).*

## 6 KEY RESEARCH ACCOMPLISHMENTS

- Hydrostatic pressure is inversely related to the change in subharmonic amplitude of Definity microbubbles *in vitro* ($r^2 = 0.79–0.99$, $p < 0.01$).
- SHAPE experiments were conducted *in vitro* in an OptiCell setup, but standing waves made results difficult to reproduce and a new, improved setup has been designed.

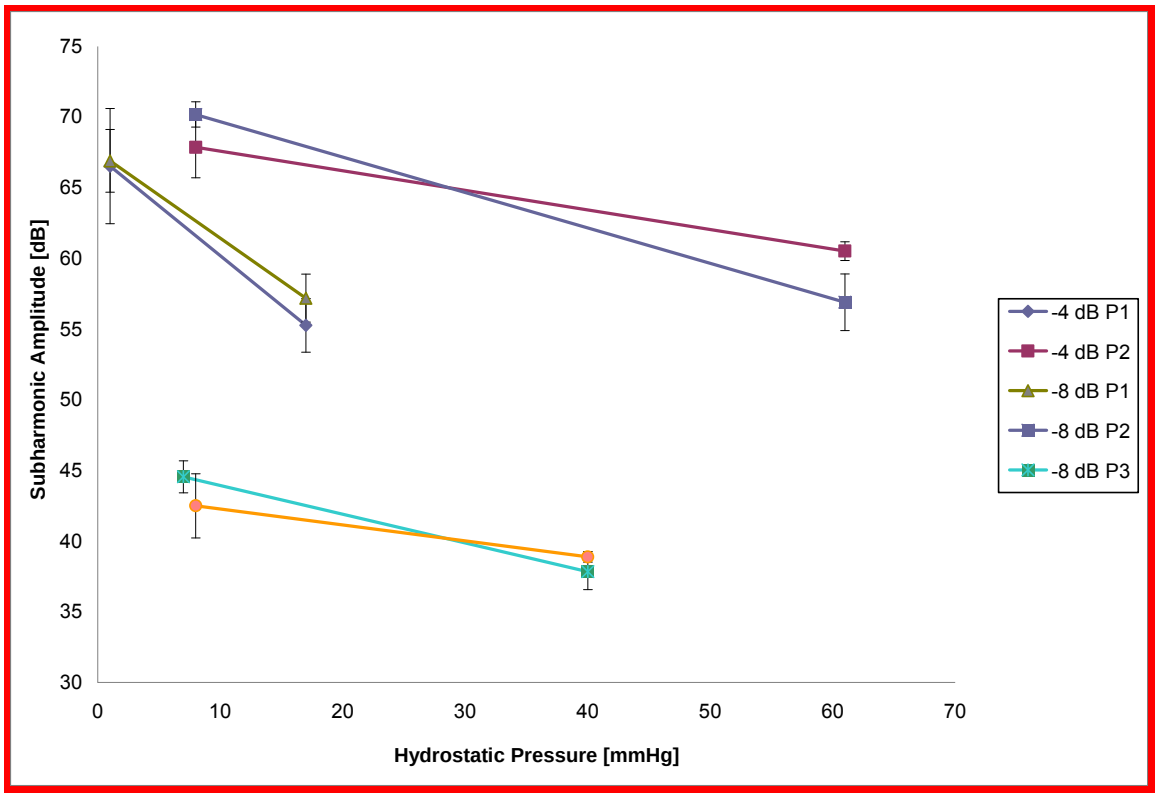


***Figure 3**: In vivo change in subharmonics as a function of IFP. While the slopes are almost identical in 2 swine, there are clearly large animal to animal variations.*

- A computer model to simulate the behavior of microbubbles as a function of pressure has been further developed.
- Software for processing of *in vivo* SHAPE data has been optimized.
- *In vivo* proof of concept for SHAPE as a noninvasive monitor of IFP has been provided.
- The L9-4 linear array has been selected for the rat xenograft studies.

## 7 REPORTABLE OUTCOMES

*Publications*

V. G. Halldorsdottir, L. M. Leodore, B. Cavanaugh, F. Forsberg. Initial *in vitro* study of US pressure measurements for monitoring neoadjuvant chemotherapy of breast cancer. *Prog. RSNA*, pp. 338, 2009.

F. Forsberg, J. Dave, V. G. Halldorsdottir, M. McDonald, J. B. Liu C. Leung, K. Dickie. Noninvasive cardiac subharmonic pressure estimation in vivo. *J Ultrasound Med*, vol 29, no 3, pp. S13, 2010.

F. Forsberg. The holy grail: ultrasound microbubbles for therapy. *J Ultrasound Med*, vol 29, no 3, pp. S17, 2010.

F. Forsberg. Subharmonic imaging and pressure estimation. *Med Phys*, vol 37, no 6, pp 3397-3398, 2010.

S. Paul, K. Sarkar, F.Forsberg. Strain-softening elasticity model of the encapsulation of an ultrasound contrast microbubble. *Am Phys Soc, Div Fluid Dynam*, http://meetings.aps.org/link/BAPS.2010.DFD.LR.1, 2010.

A. Katiyar, K. Sarkar, F.Forsberg. Subharmonic response from ultrasound contrast microbubbles for noninvasive blood pressure estimation. *Am Phys Soc*, *Div Fluid Dynam,* http://meetings.aps.org/link/BAPS.2010.DFD.HL.1, 2010.

J. Dave, V. G. Halldorsdottir, J. Eisenbrey, M. McDonald, J. B. Liu, J. Raichlen, C. Leung, K. Dickie, F. Forsberg. Subharmonic signals for noninvasive cardiac pressure estimation – initial *in vivo* experience. Accepted for publication in *JUM*, 2011

*V. G. Halldorsdottir, J. K. Dave, J. Eisenbrey, P. Machado, J. B. Liu, D. A. Merton, B. C. Cavanaugh, F. Forsberg. Subharmonic aided pressure estimation for monitoring interstitial fluid pressure in tumors: *in vitro* and *in vivo* proof of concept. Accepted for publication in *JUM*, 2011

S. Paul, A. Katiyar, K. Sarkar, D. Chatterjee, W. T. Shi, F. Forsberg. Material characterization of the encapsulation of an ultrasound contrast microbubble and its subharmonic response: strain-softening interfacial elasticity model. *J Acoust Soc Am.*, vol. 127, no. 6, pp. 3846-3857, 2010.

A. Katiyar, K. Sarkar, F. Forsberg. Modeling subharmonic response from contrast microbubbles as a function of ambient static pressure. Submitted to *J Acoust Soc Am*, 2010.

V. G. Halldorsdottir, J. K. Dave, L. M. Leodore, J. R. Eisenbrey, S. Park, A. L. Hall, Kai Thomenius, F. Forsberg. Subharmonic contrast microbubble signals for noninvasive pressure estimation under static and dynamic flow conditions. Submitted to *Ultrasonic Imaging*, 2010.

J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, J. S. Raichlen, J. B. Liu, M. E. McDonald, K. Dickie, C. Leung, F. Forsberg. Noninvasive pressure estimation of the left ventricular diastolic pressures using subharmonic emissions from microbubbles - an *in vivo* pilot study. Submitted to *Circulation*, 2010.

*Presentations*

| | |
|---|---|
| October 8, 2009 | QED Program, University City Science Center, Philadelphia, PA, USA. |

| | |
|---|---|
| | • Non-invasive pressure estimation using ultrasound. |
| October 22-23, 2009 | 24th Annual Advances in Contrast Ultrasound & ICUS Bubble Course 2009, Chicago, IL, USA.<br>• In vivo subharmonic pressure estimation. |
| November 29 - December 4, 2009 | The 95th Scientific Assembly and Annual Meeting of the Radiological Society of North America, Chicago, IL, USA.<br>• Initial in vitro study of US pressure measurements for monitoring neoadjuvant chemotherapy of breast cancer. |
| March 25 - 28, 2010 | The 55th Annual Convention of the American Institute of Ultrasound in Medicine, San Diego, CA, USA.<br>• Noninvasive cardiac subharmonic pressure estimation in vivo.<br>• The holy grail: ultrasound microbubbles for therapy |
| May 7, 2010 | Spring Symposium, the Delaware Valley Chapter of the American Association of Physicists in Medicine, Philadelphia, PA.<br>• Recent advances in ultrasound for image guided therapy. |
| July 18 - 22, 2010 | 52nd Annual Meeting of the American Association of Physicists in Medicine, Philadelphia, PA, USA.<br>• Subharmonic imaging and pressure estimation. |
| September 30 - October 1, 2010 | 25th Annual Advances in Contrast Ultrasound & ICUS Bubble Course 2010, Chicago, IL, USA.<br>• In vivo cardiac subharmonic pressure estimation. |
| November 21–23, 2010 | 63rd Annual Meeting of the American Physical Society, Division of Fluid Dynamics Long Beach, CA, USA.<br>• Strain-softening elasticity model of the encapsulation of an ultrasound contrast microbubble.<br>• Subharmonic response from ultrasound contrast microbubbles for noninvasive blood pressure estimation. |

V. G. Halldorsdottir was selected as a finalist for the AIUM 2011 Young Investigator Award based on her work in the abstract labled * above. The competition will be judged at the upcoming Annual Conference of the AIUM (in April 2011 in New York City, NY).

## 8 CONCLUSIONS

Definity showed an inverse linear rela tionship between the change in subharmonic amplitude and hydrostatic pressure ($r^2 = 0.79 – 0.99$, $p < 0.01$) over the pressure range associated with breas t tum ors (0 – 50 mmHg) when m easured *in vitro* in the OptiCell

setup. However, we discovered that standing waves markedly influenced results and made measurements impossible to reproduce. Hence, our use of the OptiCell setup had to be abandoned and a completely new tank (with extra acoustic absorbers incorporated) have been designed (currently under construction).

Our attempts to design a realistic simulation model accounting for the experimental results have been mixed and further work is ongoing. Software for analyzing RF data from the Sonix RP scanner to produce SHAPE pressure estimates has been successfully optimized and an initial publication submitted [23].

Finally, *in vivo* proof of concept for SHAPE as a noninvasive monitor of IFP ($r^2 > 0.81$, $p < 0.01$) has been provided in a swine melanoma model (at no additional expense to this grant). This work was selected for the final of the AIUM 2011 Young Investigator Award [24].

In summary, task 1 has been partially completed while task 2 is ongoing. However, due to the delay caused by the *in vitro* experiments the project is approximately 9 to 12 months behind schedule and we, therefore, intend to request a one year no cost extension.

## 9 REFERENCES


1. Favret AM, et al., Locally Advanced Breast Cancer: Is Surgery Necessary? *The Breast Journal*, 2001. **7**(2): p. 131-137.
2. Kaufmann M, et al., International Expert Panel on the Use of Primary (Preoperative) Systemic Treatment of Operable Breast Cancer: Review and Recommendations. *J Clin Oncol*, 2003. **21**(13): p. 2600-2608.
3. Guarneri V, et al. Primary systemic therapy for operable breast cancer: A review of clinical trials and perspectives. *Cancer Letters*, 2007. **248**(2): p. 175-185.
4. Esteva, FJ, Hortobagyi GN, Integration of Systemic Chemotherapy in the Management of Primary Breast Cancer. *Oncologist*, 1998. **3**(5): p. 300-313.
5. Waljee JF, Newman LA, Neoadjuvant Systemic Therapy and the Surgical Management of Breast Cancer. *Surgical Clinics of North America*, 2007. **87**(2): p. 399-415.
6. McMasters KM, Hunt KK, Neoadjuvant Chemotherapy, Locally Advanced Breast Cancer, and Quality of Life. *J Clin Oncol*, 1999. **17**(2): p. 441-.
7. Mauri D., Pavlidis N, and Ioannidis JPA, Neoadjuvant Versus Adjuvant Systemic Treatment in Breast Cancer: A Meta-Analysis. *J. Natl. Cancer Inst.*, 2005. **97**(3): p. 188-194.
8. Heldin CH, et al., High interstitial fluid pressure - an obstacle in cancer therapy. *Nat Rev Cancer*, 2004. **4**(10): p. 806-13.
9. Less JR, et al., Interstitial hypertension in human breast and colorectal tumors. *Cancer Res*, 1992. **52**(22): p. 6371-4.
10. Taghian AG, et al., Paclitaxel Decreases the Interstitial Fluid Pressure and Improves Oxygenation in Breast Cancers in Patients Treated With Neoadjuvant Chemotherapy: Clinical Implications. *J Clin Oncol*, 2005. **23**(9): p. 1951-1961.

11. Milosevic M, et al., Interstitial fluid pressure predicts survival in patients with cervix cancer independent of clinical prognostic factors and tumor oxygen measurements. *Cancer Res*, 2001. **61**(17): p. 6400-5.
12. Goldberg BB, Liu JB, Forsberg F, Ultrasound contrast agents: A review. *Ultrasound Med Biol*, 1994. **20**(4): p. 319-333.
13. Shi WT, Forsberg F, et al., Pressure dependence of subharmonic signals from contrast microbubbles. *Ultrasound Med Biol*, 1999. **25**(2): p. 275-283.
14. Ishihara K, et al., New Approach to Noninvasive Manometry Based on Pressure Dependent Resonant Shift of Elastic Microcapsules in Ultrasonic Frequency Characteristics. *Jap J Appl Phys*, 1988. **27**(Suppl. 27-1): p. 125-127.
15. Forsberg F, Shi WT, Goldberg BB, Subharmonic imaging of contrast agents. *Ultrasonics*, 2000. **38**(1-8): p. 93-98.
16. Yudina A, Lepetit-Coiffé M, Moonen CT. Evaluation of the temporal window for drug delivery following ultrasound-mediated membrane permeability enhancement. *Mol Imaging Biol*. 2010. PMID: 20521134.
17. Sarkar K, Shi WT, Chatterjee D, Forsberg F. Characterization of ultrasound contrast microbubbles using in vitro experiments and viscous and viscoelastic interface models for encapsulation. *J Acoust Soc Am*, **118**:539-550, 2005.
18. Halldorsdottir VG, Leodore LM, Cavanaugh B, Forsberg F. Pressure estimation for monitoring neoadjuvant chemotherapy of breast cancer: In vitro measurements (abstract). *Ultrasonic Imaging*, **31**: 46, 2009.
19. Halldorsdottir VG, Leodore LM, Cavanaugh B, Forsberg F. Initial in vitro study of US pressure measurements for monitoring neoadjuvant chemotherapy of breast cancer (abstract). *Prog. RSNA*, 338, 2009.
20. Paul S, Katiyar A, Sarkar K, Chatterjee D, Shi WT, Forsberg F. Material characterization of the encapsulation of an ultrasound contrast microbubble and its subharmonic response: strain-softening interfacial elasticity model. *J Acoust Soc Am.*, vol. 127, no. 6, pp. 3846-3857, 2010.
21. Katiyar A, Sarkar K, Forsberg F. Modeling subharmonic response from contrast microbubbles as a function of ambient static pressure. Submitted to *J Acoust Soc Am,* 2010.
22. Forsberg, Halldorsdottir VG, Dave J, McDonald M, Liu JB, Leung C, Dickie K. In vivo noninvasive cardi iac subharmonic pressure estimation (abstract). *Ultrasonic Imaging*, **31**:45-46, 2009.
23. Dave JK, Halldorsdottir VG, Eisenbrey JR, Raichlen JS, Liu JB, McDonald ME, Dickie K, Leung C, Forsberg F. Noninvasive pressure estimation of the left ventricular diastolic pressures using subharmonic emissions from microbubbles - an in vivo pilot study. Submitted to *Circulation*, 2010.
24. Halldorsdottir VG, Dave JK, Eisenbrey J, Machado P, Liu JB, Merton DA, Cavanaugh BC, Forsberg F. Subharmonic aided pressure estimation for monitoring interstitial fluid pressure in tumors: in vitro and in vivo proof of concept (abstract). Accepted for publication in *J Ultrasound Med*, 2011

## Appendix I

The Statement of Work from the original proposal:

**Objective 1**
**Task 1:** Computer modeling and *in vitro* experiments (months 1 - 6)

a. Construct an *in vitro* experimental pulse-echo system for investigating the effect of hydrostatic pressure variations on contrast microbubbles and measuring the resulting changes in backscattering (Month 1).
b. Design and modify numerical codes for a theoretical model describing the dynamics of contrast microbubbles under different pressure conditions (Months 1 - 3).
c. Calculate the behavior of individual contrast microbubble and the collective behavior of contrast microbubble populations (Months 3 - 6).
d. Measure changes in backscattered fundamental, second and subharmonic signals for different contrast agents as a function of pressure (Months 2 - 6).
e. Predict optimal contrast agents for SHAPE according to the numerical simulations (Month 6).
f. Select optimal contrast agent(s) for SHI and SHAPE. The selection will mainly be based on experimental measurements (Month 6).

**Objectives 2 - 3**
**Task 2:** Design and implementation of SHAPE on a commercial US scanner (months 7 - 12)

a. Optimize SHI and SHAPE, based on *in vitro* measurements and simulations using the actual parameters of the designated transducers (Months 7 – 8).
b. Modify a state-of-the-art US imaging system (the Sonix RP) to incorporate the SHI contrast imaging modality and to perform SHAPE (Months 8 - 10).
c. Evaluate the new imaging modality and SHAPE in an *in vitro* phantom using the modified US scanner (Months 11 - 12).
d. Prepare regulatory review and obtain approval for animal studies (Months 9 - 12).

**Objectives 3 - 5**
**Task 3:** Animal experiments, data collection and analysis (months 13- 36)

a. Create and grow breast tumors by implanting one of three human breast cancer cell lines (SKBR3, BT474 or MCF-7) into the mammary fat pad of athymic, nude rats (Months 13 - 34).
b. Calibrate *in vivo* SHAPE results based on IFP measurements obtained with the intra-compartmental pressure monitor in 21 nude rats. Three groups (one per cell line) of 7 rats with breast tumors implanted will be studied (months 14 - 16).

c. Produce and evaluate the ability of SHI to depict normal vascularity as well as breast tumor angiogenesis in human xenografts implanted in nude rats compared to CD31 stained specimens (Months 17 - 34).
d. Validate the clinical potential of SHAPE as a therapy monitoring tool by studying 180 human xenograft breast tumors in nude rats (42 normal rats and 138 after administration of a chemotherapy agent paclitaxel) and comparing results to intra-compartmental pressure measurements (months 17 - 34).
e. Perform statistical analyses and write final report (months 34 - 36).